

Effect of Age on EAG Response and Attraction of Female *Anastrepha suspensa* (Diptera: Tephritidae) to Ammonia and Carbon Dioxide

PAUL E. KENDRA,¹ WAYNE S. MONTGOMERY, DANIEL M. MATEO, HELENA PUCHE,
NANCY D. EPSKY, AND ROBERT R. HEATH

USDA-ARS, Subtropical Horticulture Research Station, 13601 Old Cutler Rd., Miami, FL 33158

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ABSTRACT Current ammonia-based lures vary considerably in their ability to attract *Anastrepha* fruit flies in the field. This report presents results from electroantennography (EAG) and behavioral bioassays that examined the effect of age on fly response to ammonia and carbon dioxide, two volatile chemicals released from commercial ammonium bicarbonate lures. EAG measurements from female Caribbean fruit flies, *Anastrepha suspensa* (Loew), showed that ammonia generated a greater EAG response in sexually immature females compared with mature females. Conversely, carbon dioxide elicited stronger EAG responses in sexually mature females. In flight tunnel bioassays, females from both age groups were captured in response to ammonia ranging from 60 to 3840 $\mu\text{g/h}$, but captures declined with increasing ammonia concentration. In bioassays with the two highest ammonia release rates, captures of immature females were significantly lower than captures of mature females. Carbon dioxide, ranging from 300 to 7200 $\mu\text{g/h}$, did not capture any flies when presented alone in the flight tunnel bioassay. However, for sexually mature flies, carbon dioxide in combination with ammonia was more attractive than ammonia alone. These age-related differences in response to ammonia and carbon dioxide may account for some of the variability observed in field tests with ammonium bicarbonate lures.

KEY WORDS *Anastrepha suspensa*, electroantennogram, ammonia, carbon dioxide, synthetic attractants

TEPHRITID FRUIT FLIES, LIKE the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and several *Anastrepha* species, are major economic pests that threaten fruit and vegetable production worldwide. The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is a quarantine pest of citrus in Florida and impacts the production and marketability of guava and other tropical fruit crops (Greany and Rihard 1993). Historically, liquid protein baits have been used for detecting and monitoring *Anastrepha* populations (Steyskal 1977, Heath et al. 1993a), but identification of chemicals responsible for this attraction has increased the efficacy and decreased the costs of trapping (Heath et al. 1995, Robacker 1995). The primary attractant in synthetic lures is ammonia (Mazor et al. 1987), typically formulated as ammonium bicarbonate or ammonium acetate. Current strategies focus on the development of improved ammonia-based, female-targeted trapping systems for *Anastrepha* that are compatible with sterile male release programs. A synthetic lure consisting of ammonium acetate, putrescine, and tri-

methylamine is highly effective for capturing female *C. capitata* (Heath et al. 1997, Epsky et al. 1999), especially for detection of very low populations (Katsoyanos et al. 1999). Field studies have shown that traps baited with ammonium acetate and putrescine capture *Anastrepha* species (Heath et al. 1995, Thomas et al. 2001); however, numbers captured relative to traps baited with liquid protein have been variable (Epsky et al. 2005).

The variation observed in *Anastrepha* capture in traps baited with ammonia-emitting substrates prompted research to identify factors responsible for the variability. In a previous report (Kendra et al. 2005), electroantennography (EAG) was used to quantify female and male *A. suspensa* antennal sensitivity to ammonia and carbon dioxide, two volatiles released from commercial ammonium bicarbonate lures. In this paper, we examined the effect of female age on EAG response to ammonia and carbon dioxide and compared EAG response with behavioral response to these chemicals alone and in combination using flight tunnel bioassays.

Materials and Methods

Insects. *Anastrepha suspensa* were obtained from a laboratory colony maintained at the USDA-ARS, Sub-

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¹ Corresponding author, e-mail: pkendra@saa.ars.usda.gov.

tropical Horticulture Research Station, Miami, FL. Rearing conditions consisted of a 12:12 h (L:D) photoperiod, 70% RH, and ambient room temperature. In preparation for laboratory experiments, pupae (13 d) were removed from the colony, placed on plastic weighing trays, and held in screen cages (30 by 30 by 30 cm; Bioquip Products, Gardena, CA). Once adults began to emerge, pupal trays were transferred to new cages every 24 h until emergence ceased (3–4 d). This process resulted in mixed-sex cages containing flies of known age, staged at 1-d intervals. Adult flies were given unlimited access to water (released from agar blocks) and food (4:1 mixture of refined cane sugar: yeast hydrolysate) until they were collected for experimentation. Only female flies ranging in age from 1 to 14 d after eclosion were used in this study.

Sexual maturity of the flies was determined by dissecting females 1–14 d old ($n = 5/\text{d}$) and measuring the lengths and widths of both ovaries with a microscale (to 0.1 mm; Minitool, Los Gatos, CA). Mean ovary length multiplied by width provided an ovarian index for assessing sexual maturation (Landolt and Davis-Hernandez 1993). These ovary measurements were taken from the same flies used in the carbon dioxide EAG experiments to provide a direct comparison between fly age and EAG response.

Sample Preparation for EAG. Anhydrous ammonia gas (99.99% pure) and carbon dioxide (99.8% pure) were obtained from gas cylinders purchased from Aldrich (Milwaukee, WI). Sample dilutions were prepared at room temperature (24°C) and ambient pressure in 250-ml gas-tight glass cells containing a vent valve on one end and a septum port on the opposite end (Analytical Research Systems, Gainesville, FL). Cells were filled with air purified by using a Whatman 75–52 FTIR purge gas generator ($\text{CO}_2 < 1 \text{ ppm}$), and a Hankison HIT-20 air dryer for water removal. Dilutions were prepared by first extracting the desired volume of air from the cell, and then substituting it with the corresponding volume of pure gas. Final preparations of ammonia and carbon dioxide were 1/16 dilutions of pure gas, which yielded 62,500 ppm (vol:vol). EAG test stimuli consisted of 500- μl vapor samples, which delivered doses of 24.1 μg ammonia or 57.4 μg carbon dioxide. From published EAG dose-response curves for *A. suspensa* (Kendra et al. 2005), it was determined that these quantities of ammonia and carbon dioxide would evoke maximal EAG responses in sexually mature females. Sample quantification was performed using Fourier transform infrared (FTIR) spectroscopic analysis, as previously described (Kendra et al. 2005).

2-Butanone (99% pure; Aldrich) was used as a standard reference sample throughout all EAG tests. The standard consisted of 20 μl 2-butanone-saturated vapor ($0.53 \pm 0.03 \mu\text{g}/\mu\text{l}$; R.R.H., unpublished data), collected at room temperature from the headspace of a gas-tight vial with septum lid (Chromacol, Trumbull, CT). 2-Butanone has been shown to elicit a consistently strong EAG response in *A. suspensa*, independent of sex and age of fly, and its purity has been

confirmed by gas chromatography (Kendra et al. 2005).

EAG Recordings. Electroantennogram signals were recorded with a Syntech EAG system (Hilversum, Netherlands), which included a probe/micromanipulator (MP-15), a data acquisition interface box (serial IDAC-232), and a stimulus air controller (CS-05). Fresh antennal preparations were mounted between micropipette electrodes using a teardrop amount of salt-free electrode gel (Spectra 360; Parker Laboratories, Fairfield, NJ) applied to the tip of each pipette. The electrodes consisted of thin-walled glass capillaries (1.5 mm OD; World Precision Instruments, Sarasota, FL), filled with 0.1 N KCl and positioned over silver wires (0.5 mm; World Precision Instruments). Electroantennal signals were collected and analyzed with the Syntech EAG 2000 program on a personal computer.

A stream of humidified air, purified with activated charcoal, was passed continuously over the antennal preparation at a flow rate of 400 ml/min, with the tip of the delivery tube placed 1 mm from the antenna. The air controller was configured to allow for pulse flow compensation during sample delivery. Using gas-tight syringes (VICI Precision Sampling, Baton Rouge, LA), samples were manually injected into the airstream through a port in the delivery tube 13 cm from the tip. In each experiment, the antenna was first presented with a negative control, consisting of an injection of clean air equal in volume to the sample injections (500 μl). This was followed by injection of the standard reference compound, 2-butanone, and then with injections of test samples, either ammonia or carbon dioxide. Injections were spaced at 2-min intervals to prevent adaptation of the antenna. The negative control and the standard were presented again at the end of the experiment to assess the decline in antennal response over time. EAG responses were initially measured in millivolts (peak height of depolarization) and then normalized to the standard reference sample to correct for time-dependent variability in antennal performance. The normalized results generated by the Syntech EAG 2000 program were expressed as a percentage of the standard response. Response to the negative control was subtracted from the normalized test responses to remove any “pressure shock” caused by injection pressure. The final corrected, normalized values were used to make comparisons between individual flies and between test samples. For both compounds tested, EAG responses were measured from five insects of each age (1–14 d), and five replicate measurements were recorded from each insect. Results are presented as mean \pm SE normalized EAG responses.

Sample Preparation for Bioassays. Two sample substrates were used as sources of volatile chemicals in the flight tunnel bioassays. Substrates were placed in 500-ml flasks fitted with an entry port for the external air supply and an effluent port for delivery of volatiles into the tunnels (Analytical Research Systems, Gainesville, FL). Ammonia was released from a 100-ml aliquot of stock solution ammonium hydroxide,

NH_4OH (28–30%; Aldrich) diluted 1:800 with distilled H_2O . Concurrent release of ammonia and carbon dioxide was obtained from a commercial ammonium bicarbonate lure (AgriSense-BCS, Mid Glamorgan, UK). The lure was vented for 48 h before use to stabilize the release of volatiles. The third test sample, carbon dioxide (industrial grade, 99.5% pure), was obtained directly from a gas cylinder (PraxAir, Miami, FL).

Release rates of ammonia and carbon dioxide from substrates were determined by FTIR analysis. The system consisted of a Thermo Nicolet Magna 550II FTIR spectrometer (Thermo Electron, Madison, WI) equipped with a mercury cadmium telluride (MCT-A) detector, a KBr beamsplitter, and a 2-m (200 ml) gas cell with a thermal jacket (Thermo Electron). Anhydrous ammonia, 99.99% pure, and carbon dioxide, 99.8% pure (Aldrich), were used as standards for calibration. Digital mass flow controllers (Alicat Scientific) were used to regulate flow rate of FTIR purge-gas over the substrate source. For ammonia, purge-gas consisted of air purified with a Whatman 75–52 FTIR purge-gas generator. FTIR carrier gas for carbon dioxide was 5.0 ultra-high purity compressed helium (PraxAir) that was filtered through an Excelsorb He purifier (Supelco, Bellefonte, PA) to reduce CO_2 to <1 ppm. Resulting effluent was incorporated into a stream of FTIR purge-gas such that the total flow delivered to the gas cell was 1 liter/min. A series of different flow rates over the source was applied, and absorbance values for the effluent were measured and quantified. Absorbance range consisted of bands of $997\text{--}987\text{ cm}^{-1}$ for ammonia and $497\text{--}235\text{ cm}^{-1}$ for carbon dioxide. Quantification yielded conversion factors for determining ammonia and carbon dioxide release rates from known flow rates. From these values, controlled rates of delivery of ammonia and carbon dioxide to the wind tunnel were achieved by varying the rate of airflow over the substrate.

The volatile chemical delivery system consisted of a bank of flow meters (Aalborg, Monsey, NY) used to set the flow rate of purified air. The range of flow rates used was 25–350, 50–500, and 200–1,200 ml/min. Each sample delivered to the wind tunnel was controlled by two parallel flow meters. One meter regulated airflow to the sample flask, which determined the quantity of volatile released from the substrate. The other meter regulated flow of make-up air, which joined the line of sample effluent before introduction into the wind tunnel. The final flow rate, combining effluent and make-up air, was set to 1 liter/min for each test.

Flight Tunnel Bioassays. Bioassays were conducted as two-choice tests by using 30.2 by 30.2 by 122-cm Plexiglas flight tunnels (Heath et al. 1993b) with a flow rate of 1 liter/min. Results from these bioassays have shown good correlations with results from field tests (Epsky et al. 1993). Each tunnel had two traps made from clear plastic, snap-cap vials (140 ml; BioQuip, Gardena, CA) mounted to the inner wall of the upwind side of the tunnel. Volatile test samples were introduced into the tunnel through the back of the trap. The outside face of the trap (the snap-on cap)

was covered with green tape to provide a visual cue, and it contained a 1.5-cm-diameter hole in the center to provide a point source release of test samples and a point of entry for the flies. The interior floor of the trap was covered with fluorescent green sticky paper (Atlantic Paste and Glue Co., Brooklyn, NY) to retain flies entering the trap. Two parallel wind tunnels were used for each test run: one for sexually mature flies (10–13 d) and one for immature flies (3–6 d). Determination of maturity status was based on results obtained from ovary dissections. The positions of the two test samples within a tunnel, and the positions of the two age groups between tunnels, were switched between runs to reduce position effects. A complete test consisted of three sets of runs using the four different position combinations, giving a total of 12 replicate trials for each two-choice bioassay. For each trial, 20 females were released at the downwind end of the tunnel, and the number of flies captured inside the traps was recorded after 8 h. Tests were conducted from ≈ 0830 to 1630 hours each day, under a combination of fluorescent and natural day light.

The first bioassay evaluated female response to ammonia. Eight release rates were examined, presented as four sets of two-choice tests: 0 versus 60 $\mu\text{g/h}$, 120 versus 480 $\mu\text{g/h}$, 240 versus 960 $\mu\text{g/h}$, and 1,920 versus 3,840 $\mu\text{g/h}$. This range of ammonia bracketed experimental release rates reported as attractant from field lures (100–200 $\mu\text{g/h}$; Heath et al. 1995) and from avian feces, a natural protein food source (240–780 $\mu\text{g/h}$; Epsky et al. 1997). The second bioassay tested female response to carbon dioxide presented in three sets: 300 versus 900 $\mu\text{g/h}$, 900 versus 3,600 $\mu\text{g/h}$, and 1,800 versus 7,200 $\mu\text{g/h}$. The carbon dioxide range was chosen to be approximately equi-molar to the ammonia range tested in the first bioassay, because field lures of ammonium bicarbonate (NH_4HCO_3) were expected to liberate carbon dioxide (CO_2) and ammonia (NH_3) in a 1:1 molar ratio (equivalent to a 1:2.59 g weight ratio). The third bioassay evaluated female response to carbon dioxide when presented in combination with ammonia. To match levels reported as attractant in other tephritid species (Stange 1999), carbon dioxide release rate was set to a very low level for this test. Ammonia was delivered at a rate which maintained a 1:1 molar ratio. The third bioassay gave females a choice between ammonia (8 $\mu\text{g/h}$) and a mixture of ammonia (8 $\mu\text{g/h}$) plus carbon dioxide (20.7 $\mu\text{g/h}$).

Statistical Analysis. Regression analyses were used to describe the relationships between fly chronological age and (1) ovary index, (2) EAG response to ammonia, and (3) EAG response to carbon dioxide (SigmaPlot 8.0; SPSS, Chicago, IL). Several regression models were tested including second- and third-order polynomial, sigmoidal, hyperbolic, and logarithmic models. Indicator variables (Zar 1999) were used to determine the line to either side of a hypothetical age break point where the regression line changed slope (Capinera et al. 1987). This was done by creating a series of indicator variables that were coded with a 1 for ages to be included and with a 0 for ages to be

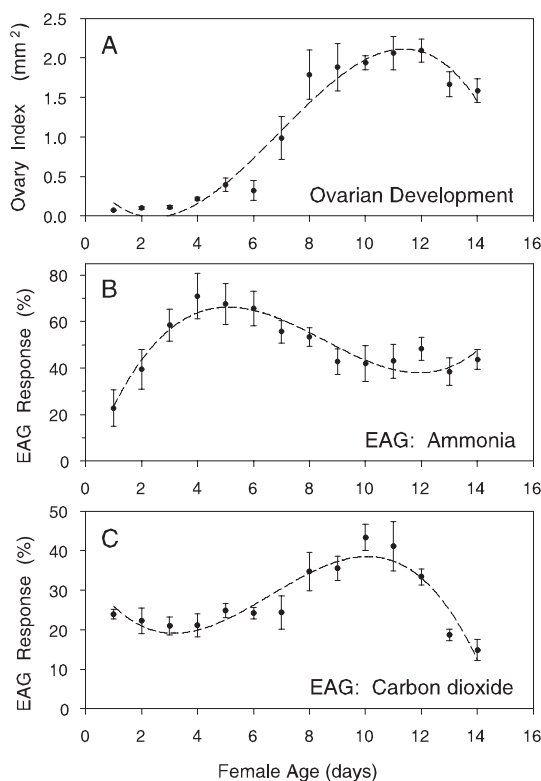


Fig. 1. Mean ovarian indices (A) and EAG responses to fixed amounts of (B) ammonia ($24.1 \mu\text{g}$) and (C) carbon dioxide ($57.4 \mu\text{g}$) for female Caribbean fruit flies measured 1–14 d after emergence. Ovarian index is defined as length of ovary multiplied by width of ovary. EAG response depicted as percentage of the standard response (2-butanone reference). Bars indicate SE ($n = 5$ females/d).

excluded in the analysis. For example, coding flies 1–3 d old with a 1 and all other flies with a 0 allowed comparisons between flies 3 d old or less with flies 4 d old or older. Twelve indicator variables were created, and these indicator variables were used sequentially as the classification variable in homogeneity of slopes models in Proc GLM (SAS Institute 1985) to test for differences in slopes and intercepts between the age groups created. Paired *t*-tests (SigmaPlot 8.0; SPSS) and analysis of variance (ANOVA) using Proc GLM were used for comparisons for factors with two or more levels, respectively.

Results

Ovarian Development. Third-order polynomial regression best described the changes in ovarian index that occurred over time ($y = 0.55 - 0.5x + 0.12x^2 - 0.01x^3$; $R^2 = 0.954$; x is fly age in days, y is ovarian index in mm^2 ; Fig. 1A). The ovaries of teneral females were very small, consisted of previtellogenic ovarioles, and increased in size only slightly within the first 4–5 d. From 6–8 d, vitellogenesis was apparent, accompanied by a rapid increase in ovary size, initially in length

and then in width. Tests with the indicator variables found that the break point for ovarian index occurred at 8 d ($F = 72.92$; $\text{df} = 3, 66$; $P < 0.0001$), and there were differences in both intercept ($F = -5.42$; $\text{df} = 1, 66$; $P < 0.0001$) and slope ($F = 4.88$; $\text{df} = 1, 66$; $P < 0.0001$) for the regression lines for flies 1–8 d old versus flies 9 d and older. Based on analysis of ovarian index, females were not considered sexually mature until at least 8 d old. The pattern of ovary development was similar to that measured by Landolt and Davis-Hernandez (1993), but peak increase in our study occurred 1 d later.

EAG Responses. A third-order polynomial regression model was also the best fit for EAG response to a fixed amount ($24.1 \mu\text{g}$) of ammonia ($y = -4.86 + 32.53x - 4.56x^2 + 0.18x^3$; $R^2 = 0.877$; x is fly age in days, y is mean normalized percent EAG response compared with the standard 2-butanone; Fig. 1B). The antennal response was low initially, increased to reach its maximum by 4–5 d, and then declined to an intermediate level maintained from 9 to 14 d. Tests with the indicator variables found that the break point in the regression line occurred at 4 d ($F = 13.54$; $\text{df} = 3, 66$; $P < 0.0001$), and there were differences in both intercept ($F = -5.01$; $\text{df} = 1, 66$; $P < 0.0001$) and slope ($F = 6.28$; $\text{df} = 1, 66$; $P < 0.0001$) for these two groups of flies.

Similarly, a third-order polynomial regression model was the best fit for EAG response to a fixed amount ($57.4 \mu\text{g}$) of carbon dioxide ($y = 35.11 - 11.30x + 2.35x^2 - 0.12x^3$; $R^2 = 0.853$; Fig. 1C). The response was low throughout the first week, peaked at 10–11 d, and then dropped off. Tests with the indicator variables found that separation of flies that were 10 d or younger from flies that were 11 d or older gave the best fit ($F = 22.47$; $\text{df} = 3, 66$; $P < 0.0001$), and there were differences in both intercept ($F = -7.78$; $\text{df} = 1, 66$; $P < 0.0001$) and slope ($F = 7.84$; $\text{df} = 1, 66$; $P < 0.0001$) for these two groups of flies.

Flight Tunnel Bioassays. Selection of mature and immature cohorts for the behavioral assays was determined a posteriori from the results obtained with the ovary dissections and EAG recordings. Ovary measurements indicated that flies that were 7–8 d old were in transition, and both immature and mature flies were represented at that stage. Therefore, to optimize separation of two groups based on maturity status, females of this transitional age were excluded from the bioassays. Sexually mature females were represented by flies 10–13 d old and immature females by flies 3–6 d old. To confirm that flies from these two cohorts differed in EAG response to both ammonia and carbon dioxide, data from these age groups were reanalyzed by *t*-test. Mean EAG response ($\pm \text{SE}$) to ammonia was $42.89 \pm 3.13\%$ for mature females versus $65.62 \pm 3.93\%$ for immature females ($t = 4.524$, $\text{df} = 38$, $P < 0.0001$); response to carbon dioxide was $34.13 \pm 2.80\%$ for mature females versus $22.74 \pm 1.08\%$ for immature females ($t = -3.791$, $\text{df} = 38$, $P = 0.0005$).

Data from the ammonia bioassays (Table 1) indicated that both sexually immature and mature females were attracted to ammonia at the lowest release rate

Table 1. Mean capture \pm SE of female *A. suspensa* in flight tunnels in response to ammonia presented in two-choice bioassays

Lower choice		Higher choice		t	df	P
Rate ($\mu\text{g/h}$)	Response	Rate ($\mu\text{g/h}$)	Response			
Immature						
0	0.0 ± 0.00	60	2.3 ± 0.45	-5.0455	11	0.0004 ^a
120	1.9 ± 0.34	480	0.8 ± 0.28	2.4609	11	0.0316 ^a
240	1.6 ± 0.29	960	0.3 ± 0.19	3.0446	11	0.0112 ^a
1,920	0.3 ± 0.14	3,840	0.5 ± 0.19	-0.8044	11	0.4382
Mature						
0	0.2 ± 0.11	60	2.5 ± 0.47	-4.5531	11	0.0008 ^a
120	2.2 ± 0.49	480	1.8 ± 0.39	1.1639	11	0.2691
240	1.6 ± 0.42	960	1.9 ± 0.42	-0.6325	11	0.5400
1,920	1.2 ± 0.27	3,840	0.9 ± 0.31	0.5404	11	0.5997

Mean response based on 20 females per trial, 12 replicate trials per choice test.
^aMeans are significantly different (paired *t*-test).

(60 $\mu\text{g/h}$) compared with the control (0 $\mu\text{g/h}$). For the remaining choice tests, immature flies were captured more often by the lower ammonia choice; however, in the test with the two highest delivery rates, few flies were caught in either trap. Mature females were captured in approximately equal numbers when provided a choice between any two ammonia concentrations. To compare results between the two age groups, the fly captures in both traps of each treatment pair were summed for each test, and the summed release rate of ammonia from paired traps was calculated per tunnel per test. Two-factor ANOVA with interaction was used to determine effects of sexual maturity (two levels) and summed ammonia release rate (four levels) on summed fly captures. Effects of both sexual maturity ($F = 10.73$; $\text{df} = 1,88$; $P = 0.0015$) and ammonia release ($F = 5.99$; $\text{df} = 3,88$; $P = 0.0009$) were significant, but the interaction was not ($F = 0.74$; $\text{df} = 3,88$; $P = 0.5323$). Capture of mature females tended to be higher than capture of immature females at all ammonia concentrations, but the differences were only significant at the highest two ammonia levels ($t = -2.549$, $\text{df} = 11$, $P = 0.0270$ and $t = -5.000$, $\text{df} = 11$, $P = 0.0004$, respectively; Fig. 2).

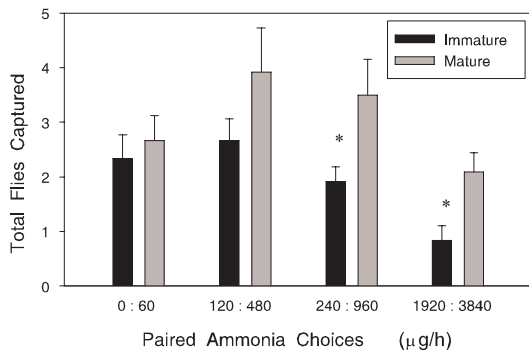


Fig. 2. Composite results of ammonia two-choice bioassays with sexually immature (3–6 d) and mature (10–13 d) female Caribbean fruit flies. Mean total flies captured per trial are shown for each of the four ammonia-choice tests. Bars indicate SE ($n = 12$ trials/choice test; 20 females released/trial). *Means are significantly different (*t*-test, $P < 0.05$).

No flies were captured in response to carbon dioxide alone in the flight tunnel bioassays. However, when presented with ammonia, carbon dioxide increased capture for sexually mature females ($t = -3.0794$, $\text{df} = 11$, $P = 0.0105$), but not for immature females ($t = 0.8044$, $\text{df} = 11$, $P = 0.4382$) compared with ammonia alone (Fig. 3).

Discussion

The EAG tests and behavioral bioassays suggest that, in relative terms, females of *A. suspensa* are more responsive to ammonia when sexually immature and more responsive to carbon dioxide when mature. These findings are consistent with the functional roles reported for these two volatile chemicals. Unlike many other insects, adult females of the higher Diptera are generally anautogenous, requiring a protein meal for oogenesis (Wheeler 1996). Ammonia is perceived by tephritid flies as a cue for protein-rich food sources, such as bacteria and avian fecal material (Bateman and Morton 1981, Drew et al. 1983, Epsky et al. 1997, 1998). In our study, peak antennal response to ammonia (4–6 d) coincides with the age of peak protein consumption reported for *A. suspensa* (Landolt and Davis-

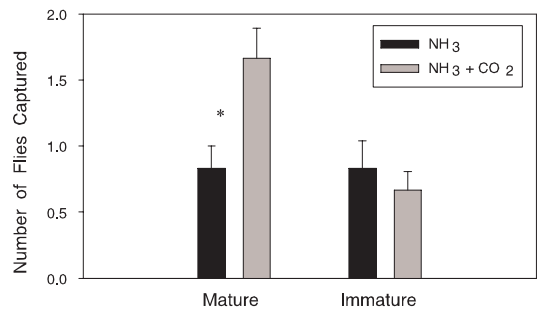


Fig. 3. Mean number of flies captured in two-choice bioassay presenting ammonia or a mixture of ammonia plus carbon dioxide to sexually mature (10–13 d) and immature (3–6 d) female Caribbean fruit flies. Bars indicate SE ($n = 12$ trials/age class; 20 females released/trial). *Means are significantly different (*t*-test, $P < 0.05$).

Hernandez 1993). This EAG peak immediately preceded the rapid increase in ovary development (6–8 d), but once the ovaries were fully developed (8–12 d), the antennal response diminished. This pattern in EAG response to ammonia would probably be cyclical if, after the initial period of oviposition, a female required additional protein consumption for additional egg production.

Behaviorally, ammonia attracted both immature and mature females, but there were differences in response related to ammonia release rate. A decrease in captures with increasing concentration suggested that ammonia at high levels may be less attractive or possibly repellent for *A. suspensa*. At the two highest ammonia release rates, captures of immature females were significantly lower than captures of mature females. This correlates with the stronger EAG response elicited by ammonia from immature flies. Increased antennal receptor sensitivity to ammonia could result in lower thresholds for detection, and thus, high doses may be less attractive to immature flies compared with mature ones. With *C. capitata*, captures of unmated (immature) females in the field declined with increasing doses of ammonium acetate lures (Heath et al. 1995).

Carbon dioxide, a metabolic by-product of respiration, is well known as a host-seeking cue for hematophagous Diptera (Bowden 1991, Willemse and Takken 1994). Stange (1999) showed that carbon dioxide may also play a functional role in tephritid host orientation. From experimental work with the Queensland fruit fly, *Bactrocera tryoni* (Froggatt), she suggested that gravid females may detect small fluctuations in carbon dioxide concentration occurring in the vicinity of ripening host fruits and locate surface lesions (point sources of carbon dioxide release) as suitable sites for oviposition. The higher EAG response to carbon dioxide recorded from sexually mature female *A. suspensa* is consistent with this theory. In addition, EAG response to carbon dioxide is significantly greater in females than in males, and females can detect lower concentrations of carbon dioxide (Kendra et al. 2005).

In the bioassay system, mature females responded to carbon dioxide when presented concurrently with ammonia. This was not observed with immature females, and neither cohort responded to carbon dioxide alone (at the release rates tested in the flight tunnels). It seems that carbon dioxide may function as a synergist to ammonia for sexually mature, but not for immature, female *A. suspensa*. Previous EAG studies (Kendra et al. 2005) documented that antennal response to a mixture of ammonia and carbon dioxide is the summation of individual responses to the two compounds. This additive effect suggests that two separate olfactory receptors may be involved in the detection of ammonia and carbon dioxide by *A. suspensa*, as has been reported for *B. tryoni* (Hull and Cribb 2001). Stimulation of both receptors may be required before the carbon dioxide component confers additional attraction.

This study on female *A. suspensa* identified age-related differences in response to ammonia and car-

bon dioxide, both in receptor potentials measured by EAG and in attraction behaviors assessed by flight tunnel bioassays. The results suggest that age structure of a fruit fly population may influence field captures in traps baited with ammonia-based lures. Higher ammonia release rates and the presence of carbon dioxide may favor capture of sexually mature females. Further study is needed to determine if these age effects are the result of physiological changes resulting from sexual maturation or from successful mating of female *A. suspensa*. Possible mechanisms to account for the differences are beyond the scope of this study, but may include hormone-mediated temporal regulation of the olfactory receptors or odorant binding proteins within the antenna and central nervous system processing within the antennal lobe or higher centers. Despite the complexity of this system, an approach using quantitative EAG and behavioral bioassays may facilitate identification of major factors influencing fruit fly attraction to chemical lures. An understanding of these factors—including sex, age, sexual maturation, mating status, nutritional requirements, dose effects, and contributions of multiple components—will aid in interpretation of variable field results and advance the development of improved lures for tropical fruit fly pests.

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